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Enhanced Mineralization of [^{14}C]Atrazine in *Kochia scoparia* Rhizospheric Soil from a Pesticide-Contaminated Site

Brenda S. Perkovich,* Todd A. Anderson, ‡ Ellen L. Kruger & Joel R. Coats

Pesticide Toxicology Laboratory, Department of Entomology, Iowa State University, Ames, Iowa 50011-3140, USA

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Abstract: Mineralization of atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) in soil treated with a mixture of atrazine and metolachlor (2-chloro-6′-ethyl-*N*-(2-methoxy-1-methylethyl)acet-*o*-toluidide at concentrations typical of point-source contamination (50 $\mu g\ g^{-1}$ each) was significantly greater ($P < 0{\cdot}001$) in rhizospheric soil from *Kochia scoparia* (L.) Roth., a herbicide-resistant plant, than in non-vegetated and control soils. Soils were collected from an agrochemical dealership contaminated with several herbicides, including atrazine, metolachlor, trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine and pendimethalin (*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidene), at concentrations well exceeding the field application rates. Mineralization rates of ring-labeled atrazine in both rhizospheric and non-vegetated soils were quite high (>47% of the initial ^{14}C applied after 36 days) compared to literature values. These results suggest that plants such as *Kochia* might be managed at pesticide-contaminated sites to help facilitate microbial degradation of wastes such as atrazine in soil.

Key words: atrazine, rhizosphere, pesticide, phytoremediation.

1 INTRODUCTION

The widespread use of pesticides in agriculture during the last 40 years has stimulated the growth of retail agrochemical dealerships. Unfortunately, many of these dealerships have experienced soil and water contamination problems from normal operating procedures and accidents.[1–3] In most instances, the costs associated with current cleanup technologies preclude their use at these facilities.[2] Biological remediation is attractive for these sites and others with similar contamination problems because of its favorable economics and potential for treatment in situ.[2]

The use of plants to facilitate bioremediation of chemically contaminated soil has been proposed[4,5] and is a potential technology for overcoming some of the inherent limitations to biological cleanup approaches, such as inadequate microbial populations or inadequate microbial activity. Plants encourage the proliferation of micro-organisms in surface soils by exuding carbohydrates and amino acids into the root zone.[6] The overall effect of the plant–micro-organism interaction is an increase in microbial biomass by an order of magnitude or more over microbial biomass in non-vegetated soils.[7,8] The root zone of plants also appears to provide an environment conducive to co-metabolism, leading to increased degradation of xenobiotic organic compounds by this process in the rhizosphere.[9] The synergistic interactions of the microbial community in the rhizosphere can also facilitate degradation of recalcitrant compounds.[10]

At pesticide-contaminated sites, vegetation may be inhibited by the presence of herbicides. Nonetheless, herbicide-resistant plants exist at these sites, and rhizospheric soils from these plants have previously shown the ability to degrade mixtures of herbicides.[11] In addition, previous studies[10,12] on the herbicide-degradative capability of rhizospheric soils of other (non-herbicide-resistant) plant species support the use of vegetation in remediating pesticide-contaminated sites.

* Present address: School of the Environment, Duke University, Durham, NC 27708.
‡ To whom correspondence should be addressed.

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Atrazine (6-chloro-N^2ethyl-N^4- isopropyl-1,3,5-triazine-2,4-diamine) is moderately persistent in the environment [$t_{1/2}$ = 37–168 days][13] and, to our knowledge, there have been no documented instances of atrazine failing to control weed species because of enhanced degradation (adaptation of microbial populations due to frequent or repeated pesticide applications) in soil, despite widespread use of this herbicide for decades. Mineralization of atrazine, particularly the triazine ring, is relatively rare and typically does not occur at a rapid rate.[14] However, recent research with a mixed microbial culture isolated from a pesticide-contaminated site indicated that ring-labeled atrazine at high concentrations (100 $\mu g\ g^{-1}$) could be mineralized quite rapidly in culture media [$t_{1/2}$ = 0·5–8 days].[15]

The purpose of the study reported here was to determine whether rhizospheric soil from a pesticide-contaminated site could mineralize atrazine in a herbicide mixture at concentrations typical of point-source contamination (>10x field application rate). Soils (rhizospheric and non-vegetated) were collected from a site contaminated with several commercial pesticides and spiked with a mixture of atrazine and metolachlor at 50 $\mu g\ g^{-1}$ soil each. Although laboratory experiments such as these can be complicated by the physical, chemical, and biological heterogeneity of actual environmental samples, they are valuable for assessing the ability of micro-organisms to perform in situations that most closely mimic contaminated sites. Evaluation of rhizosphere soils for pesticide degradation capabilities in studies such as these should be valuable for selecting appropriate plant species for use in remediation of contaminated sites.

2 MATERIALS AND METHODS

2.1 Soil samples

The soils for this study were obtained from an agrochemical dealership in central Iowa. Soil samples were excavated from the top 15 cm (A horizon) at various locations at the site. The rhizospheric soils were obtained from the root zone of *Kochia scoparia* (L.) Roth. vegetating the area. *K. scoparia* was chosen based on (1) its prevalence at the site, and (2) results of previous studies.[11] Samples were placed in Whirl-Pak® bags (NASCO, Fort Wilkinson, WI) and transported back to the laboratory on ice. All soil samples were sieved (2.4 mm) and stored in the dark 4°C prior to use (samples were stored for no more than 14 days). Composite samples (non-vegetated and *K. scoparia* rhizospheric soil) were made from all of the samples collected and these composite samples were analyzed for the physicochemical properties as well as pesticide contaminants. In order to determine further contaminant identity and distribution, several individual surface soil samples from the site (both non-vegetated and *K. scoparia* rhizospheric soil) were extracted and analyzed by gas chromatography as described previously.[11]

2.2 Microbial respiration

Microbial respiration was used as a method to estimate microbial biomass and activity in soils from the site, as well as to test whether high contaminant concentrations were toxic to the soil micro-organisms. Triplicate soil samples (20 g) were moistened to the gravimetric water content at −33 kPa (1/3 bar) with distilled, deionized water and incubated in the dark at 24°C in 11 × 5-cm glass jars. An infrared gas analyzer (LIRA Model 3000, Mine Safety Appliances Company, Pittsburgh, PA) was used to monitor carbon dioxide efflux[16,17] at 24-h intervals for 15 days. Carbon dioxide evolution was used to calculate microbial biomass in the composite non-vegetated and composite *K. scoparia* rhizospheric soil according to methods described in Parkinson and Paul.[18] In addition, as part of the site characterization, the respiration rates of various individual surface soil samples from the site (non-vegetated and *K. scoparia* rhizospheric soil) were also monitored, as described above, and compared to uncontaminated (as determined by gas chromatography) control soil from the site as a simple and rapid indication of contaminant toxicity to soil micro-organisms.[19]

2.3 Mineralization studies

In order to determine mineralization attributes of the indigenous soil micro-organisms, soils were treated with ^{14}C-labeled herbicide. Three composite samples each of non-vegetated soil, *K. scoparia* rhizospheric soil, and sterile control soil (*K. scoparia* rhizospheric soil autoclaved for 1 h on three consecutive one-half day intervals) were treated with a mixture of atrazine and metolachlor. Soil samples were spiked with 50 $\mu g\ g^{-1}$ [*U-ring*-^{14}C]atrazine (98% radiochemical purity) and 50 $\mu g\ g^{-1}$ unlabeled metolachlor (2-chloro-6′-ethyl-*N*-(2-methoxy-1-methylethyl)acet-*o*-toluidide. For each treatment, time zero and incubation replicates were prepared by adding 45 g soil to 230 ml French square bottles. Incubation replicate soils were moistened to the gravimetric water content at −33 kPa (1/3 bar) with distilled, deionized water. Each bottle was equipped with a 20 ml vial containing sodium hydroxide (10 ml, 0·1 M) to trap [^{14}C]carbon dioxide and a section of polyurethane foam (suspended from the top of the bottle) to trap ^{14}C-volatiles.

The time zero samples were extracted immediately to determine recovery efficiency. For extraction, methanol + water (9 + 1 by volume) was used as previously described.[20] Subsamples of the extracts (500 μl) were added to 10 ml Ultima Gold scintillation cocktail

(Packard Instrument CO., Downers Grove, IL) and analyzed by liquid scintillation spectroscopy (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD). A quench curve was used to adjust the samples for chemical and physical quenching.

All samples were incubated in the dark at 20°C. Every third day, the sodium hydroxide traps were replaced. Duplicate subsamples from the traps (1 ml sodium hydroxide in 3 ml cocktail) were analyzed by liquid scintillation spectroscopy as described above. At the conclusion of the test period, the compartments of each sample jar were extracted. ^{14}C-volatiles were extracted from the polyurethane foam traps with 20 ml hexane. Radioactivity in subsamples of the hexane extracts (1 ml extract in 5 ml scintillation cocktail) was determined. Soils were extracted 3x with methanol + water (9 + 1 by volume) as previously described.[20] The soil extracts were pooled, and evaporated to remove the methanol from the samples. Each sample extract was diluted to 65 ml with distilled, deionized water and, in triplicate, 1 ml was added to 5 ml of cocktail and the radioactivity determined. The remainder of the extract was archived for further chemical analysis. Extracted soils were dried, and subsamples were oxidized using a sample oxidizer. Extraction data were compiled into a mass balance (total recovered radioactivity) for the ^{14}C-labeled compounds.

3 RESULTS

3.1 Soils

The physicochemical properties of the composite non-vegetated and composite *K. scoparia* rhizospheric soils were relatively similar (Table 1) considering the typical soil heterogeneity of contaminated sites. Both soils were characterized as loams, with the rhizospheric sample having a slightly higher organic matter content, silt, CEC, and pH. In addition, both soils were classified as Nicolett Webster. Residue analyses of individual soil samples (rhizosphere and non-vegetated) indicated that the primary contaminants at the site were herbicides. All of the samples tested had detectable levels of at least one herbicide. Several of the individual surface soil samples contained herbicide contaminants at concentrations well above the field application rate. Assuming uniform mixing in a 15-cm soil layer, and a soil density of 1·8 g cm^{-3}, the field application rate for all the herbicides detected would be around 1 $\mu g\ g^{-1}$. Maximum concentrations in the individual soil samples for trifluralin, atrazine, metolachlor, pendimethalin, and cyanazine were 240, 70, 120, 330 and 3 $\mu g\ g^{-1}$, respectively. The heterogeneous distribution of herbicide contaminants in the soils is characteristic of these types of site,[2] making monitoring and remediation difficult. Herbicide residues in the composite soil samples (*K. scoparia* rhizosphere and non-vegetated) were consistently $< 10\ \mu g\ g^{-1}$. Overall, the composite *K. scoparia* rhizospheric soil had lower levels of herbicide contamination than corresponding composite non-vegetated soils, suggesting possible in situ degradation of the soil contaminants, or the ability of *Kochia* to grow only in soils with low concentrations of herbicides.

3.2 Microbial activity and biomass

Despite high concentrations of herbicide contamination, microbial activity in the surface soils did not appear to be dramatically inhibited. Respiration monitoring of individual soil samples from the site (collected as part of the site characterization) indicated that increased herbicide concentrations did not implicitly lead to a corresponding decrease in carbon dioxide evolution. Initially, respiration rates for about half of the samples tested were lower than respiration in uncontaminated (herbicide concentrations below detection limit of 0·5 $\mu g\ g^{-1}$) control soil from the site. However, several of these samples recovered to control levels by the end of the experiment (15 days).

The respiration levels in the composite *K. scoparia* rhizospheric sample were initially higher compared with composite non-vegetated soil, consistent with the concept of increased microbial biomass and activity associated with the root zone. However, respiration rates in composite *Kochia* rhizospheric soil decreased after about three weeks of incubation, suggesting that prolonged absence of a living plant has a negative influence on the microbial activity. Estimates of microbial biomass were significantly greater (t-test, $P \leq 0{\cdot}001$) in composite *K. scoparia* rhizospheric soil than composite non-vegetated soil: 1460 μg biomass g^{-1} soil and 980 μg biomass g^{-1} soil, respectively.

TABLE 1
Physicochemical Properties of Composite Non-vegetated Soil and Composite *Kochia scoparia* Rhizospheric Soil used in Studies on Mineralization of [^{14}C]Atrazine

Sample	*Texture*	*Sand* (%)	*Silt* (%)	*Clay* (%)	*N* (%)	*CEC* (*meq* 100 g^{-1})	*Organic matter* (%)	*pH*
Non-vegetated	Loam	50	34	16	0·26	12·7	3·9	7·0
Kochia scoparia	Loam	32	50	18	0·22	14·1	5·0	7·5

3.3 Mineralization of [*U-ring* ^{14}C]atrazine

Overall, *K. scoparia* rhizospheric soil had a significantly greater (*t*-test, $P < 0{\cdot}001$) rate of [^{14}C]atrazine mineralization than non-vegetated soil and sterile control soil (Fig. 1). After 36 days, the rhizospheric soil mineralized an average of 62·1 (±4·3)% of the [^{14}C]atrazine initially applied, while the non-vegetated and sterile control soils averaged 48·7 (±3)% and 4·4 (±0·2)% mineralization, respectively. *Kochia* rhizospheric soil began mineralizing atrazine after only six days; a longer lag period was observed in the non-vegetated soil (12 days). However, both the rhizospheric and non-vegetated soils mineralized ring-labeled atrazine quite readily. Atrazine mineralization half-lives for *Kochia* rhizospheric and non-vegetated soil were approximately 16 and 37 days, respectively. Based on the relatively rapid mineralization half-lives of atrazine in both soils, it does not appear that the presence of metolachlor at 50 $\mu g\ g^{-1}$ had a negative influence on the degradation. A duplicate experiment incorporating a mixture of [*U-ring* ^{14}C]metolachlor and unlabeled atrazine (both at 50 $\mu g\ g^{-1}$) has been completed and data analyses are currently in progress. However, preliminary results indicate that only a small percentage (<10%) of the added metolachlor was mineralized by either the non-vegetated soil or the *K. scoparia* rhizospheric soil. Mass balances (total recovered radioactivity) for the [^{14}C]atrazine mineralization study were 85–108% for all samples.

4 DISCUSSION

Soil residue analyses indicated relatively high concentrations of herbicides at this site. Despite these high concentrations, soil microbial biomass and activity, as indicated by respiration, is not completely suppressed and could potentially lead to biological removal of the herbicide contaminants. In addition, respiration in composite *K. scoparia* rhizospheric soil was greater than respiration in composite non-vegetated soils, indicating a larger and/or more active microbial community associated with the roots of *Kochia* at the contaminated site. It should be noted that the lower herbicide concentrations initially extracted from the composite rhizospheric soil may be due to natural in situ degradation of the contaminants, or the ability of *Kochia* to grow only in soils with low concentrations of herbicides. Preliminary results of field studies by our group suggest the former, as *K. scoparia* seedlings from the contaminated site have been successfully transplanted into bulk soil containing a mixture of trifluralin, atrazine, metolachlor, and pendimethalin at 260, 170, 3, and 18 $\mu g\ g^{-1}$, respectively.

Atrazine is readily mineralized by the indigenous microbial community associated with the roots of *K. scoparia*, as indicated by the radiotracer studies. In addition, the presence of metolachlor at 50 $\mu g\ g^{-1}$ did not appear to have a negative influence on the degradation of atrazine. We have screened other soils from waste areas for their ability to mineralize high concentrations of atrazine and found positive results for rhizospheric soils from musk thistle (*Carduus nutans* L.) and catnip (*Nepeta cateria* L.), among others.[21]

Previous studies on atrazine mineralization have been equivocal. While most studies have revealed very little (<15%) mineralization,[14] recent research by Mandelbaum *et al.*,[15] using bacterial mixed cultures, indicated rapid mineralization of ring-labeled atrazine ($t_{1/2} \leqq 8$ days). The atrazine mineralization half-lives obtained in this study using environmental samples ($t_{1/2} = 16$ days for *K. scoparia* rhizospheric soil) compare favorably with the latter study.

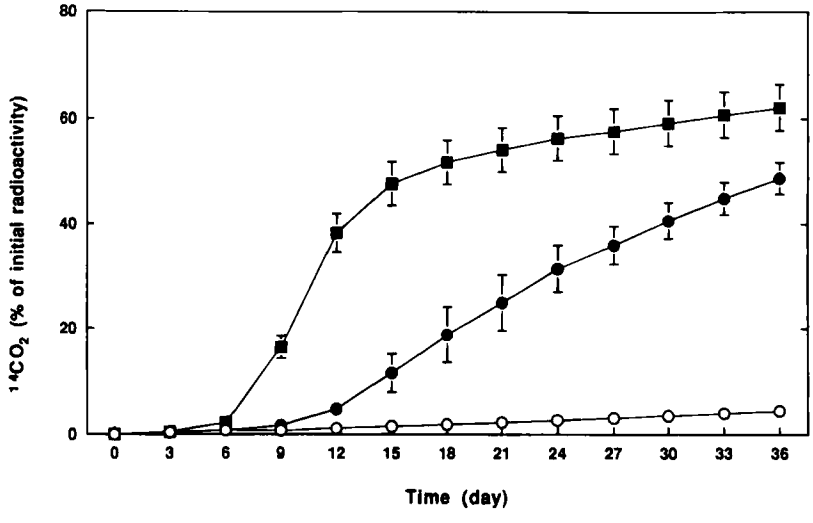

Fig. 1. Mineralization of [*U-ring*-^{14}C]atrazine in (○) sterile (autoclaved) control soils, (●) non-vegetated soils, and (■) *Kochia scoparia* rhizospheric soils from a pesticide-contaminated site. Samples were spiked with a mixture of [^{14}C]atrazine and unlabeled metolachlor at 50 $\mu g\ g^{-1}$ each. Error bars represent one standard deviation of the mean of triplicate samples.

Although the absence of a living plant in the rhizospheric samples prevented loss of ^{14}C by root uptake, slower atrazine mineralization may have resulted from the lack of rhizospheric micro-organisms, especially during the later stages of the experiment. Mineralization in both rhizospheric and non-vegetated soils leveled off near the end of the experiment, perhaps indicating that, through non-equilibrium sorption,[22] less of the atrazine is biologically available for utilization by the micro-organisms. However, the rhizospheric soils were also lacking the continuous influence of the root exudates. Thus, the differences in [^{14}C]atrazine mineralization observed between *Kochia* rhizospheric soil and non-vegetated soil in the present study may be conservative.

Non-vegetated soils also mineralized ring-labeled atrazine quite well, despite the rarity of this event in the literature. Frequent or repeated applications of some pesticides have been shown to result in enhanced degradation rates due to adaptation of the microbial populations.[23] This phenomenon has traditionally been noted to occur when such pesticides were applied at field rates. The prolonged exposure of micro-organisms in the soils used in this study to high concentrations of several hazardous chemicals (herbicides, insecticides, fumigants) has undoubtedly had a positive influence on their ability to mineralize atrazine and probably other organics.

The research presented here supports the use of rhizospheric micro-organisms associated with herbicide-resistant plants to enhance microbial degradation of atrazine in soil at contaminated sites. Naturally occurring plants, such as *K. scoparia*, have the capacity to be used as in-situ agents of bioremediation by facilitating the proliferation of micro-organisms in surface soil with the ability to mineralize high concentrations of atrazine. Further investigation of the relationship between plants and rhizospheric micro-organisms at contaminated sites will help to identify situations where the use of plants to enhance microbial degradation can be appropriate. Such identifications may ultimately provide the agrochemical industry with a more suitable technology for cleanup at a cost considerably less than that of traditional remediation methods.

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